

ABSTRACT

Cyanine and related dyes, such as merocyanine, styryl and oxonol dyes, are strongly light-absorbing and highly luminescent. Cyanine and related dyes having functional groups make them reactive with amine, hydroxy and sulfhydryl groups are covalently attached to proteins, nucleic acids, carbohydrates, sugars, cells and combinations thereof, and other biological and nonbiological materials, to make these materials fluorescent so that they can be detected. The labeled materials can then be used in assays employing excitation light sources and luminescence detectors. For example, fluorescent cyanine and related dyes can be attached to amine, hydroxy or sulfhydryl groups of avidin and to antibodies and to lectins. Thereupon, avidin labeled with cyanine type dyes can be used to quantify biotinylated materials and antibodies conjugated with cyanine-type dyes can be used to detect and measure antigens and haptens. In addition, cyanine-conjugated lectins can be used to detect specific carbohydrate groups. Also, cyanine-conjugated fragments of DNA or RNA can be used to identify the presence of complementary nucleotide sequences in DNA or RNA.

The cyanine dyes have the advantage that by synthesizing structural modifications of the chromophore portion of the molecule, different fluorescent labeling reagents can be made that will absorb and fluoresce light at many different wavelengths in the visible and near infrared region of the spectrum.

Also, the cyanine and related dyes have an advantage in their structural versatility. That is, they can be synthesized in many structural forms and with a variety of functional groups attached. This versatility permits control over such factors as the solubility of the dye and labeled

product and helps reduce nonspecific binding of the labeled material to irrelevant components in the assay mixture. This versatility also allows for selection of labeling reagents that minimally perturb the function of the labeled product.